1: Transplantation. 2001 Apr 27;71(8):1040-6.

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Attenuation of ischemia and reperfusion injury of canine livers by inhibition of type II phospholipase A2 with LY329722.

Ogata K, Jin MB, Taniguchi M, Suzuki T, Shimamura T, Kitagawa N, Magata S, Fukai M, Ishikawa H, Ono T, Furukawa H, Fujita M, Todo S.

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BACKGROUND: Membrane phospholipid breakdown, caused by ischemia and reperfusion (I/R) of the liver, releases free fatty acids including arachidonic acids and lysophospholipids, which serve as precursors of various inflammatory lipid derivatives. Phospholipase A2 (PLA2) is a key enzyme that initiates this reaction. In this study, we tested our hypothesis that a type II PLA2 inhibitor, LY329722, could attenuate hepatic I/R injury caused by a 2-hr total hepatic vascular exclusion (THVE) in dogs. METHODS: Eighteen beagle dogs, subjected to a 2-hr THVE, were divided into three groups. Group 1 (n=6) was untreated and served as a control group. LY329722 was administered to animals in group 2 (n=6) intravenously (0.2 mg x kg(-1) x hr(-1)) for 60 min before ischemia, and to animals in group 3 (n=6) for 60 min starting 15 min before reperfusion (0.2 mg x kg(-1) x hr(-1)). Animal survival, systemic and splanchnic hemodynamics, hepatic tissue blood flow, liver functions, energy metabolism, hepatic venous thromboxane B2 and endothelin-1 levels, phospholipid levels and tumor necrosis factor-a mRNA expression in liver tissue, and histopathologic findings were evaluated. RESULTS: Two-week animal survival was 33% (two of six) in group 1, and 100% (six of six) in groups 2 and 3. LY329722 improved systemic and splanchnic hemodynamics, hepatic tissue blood flow, and energy metabolism, reduced liver enzyme, thromboxane B2, and endothelin-1 release, prevented hepatic phospholipid degradation and tumor necrosis factor-alpha mRNA expression, and lessened histopathologic damage and the number of neutrophil infiltrating into the liver tissue. CONCLUSION: The present study demonstrated that a type II PLA2 inhibitor, LY329722, attenuated hepatic I/R injury caused by a 2-hr THVE model in dogs.

PMID: 11374398 [PubMed - indexed for MEDLINE]

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1: Am J Physiol Heart Circ Physiol. 2001 Jun;280(6):H2572-80.

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Ischemic-reperfused isolated working mouse hearts: membrane damage and type IIA phospholipase A2.

De Windt LJ, Willems J, Roemen TH, Coumans WA, Reneman RS, Van Der Vusse GJ, Van Bilsen M.

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For the murine heart the relationships between ischemia-reperfusion-induced loss of cardiac function, enzyme release, high-energy phosphate (HEP), and membrane phospholipid metabolism are ill-defined. Accordingly isolated ejecting murine hearts were subjected to varying periods of ischemia, whether or not followed by reperfusion. On reperfusion, hemodynamic function was almost completely restored after 10 min of ischemia [83 +/- 14% recovery of cardiac output (CO)], but was severely depressed after 15 and 20 min of ischemia (40 +/- 24 and 31 +/- 24% recovery of CO, respectively). Reperfusion was associated with partial recovery of HEP stores and enhanced degradation of phospholipids as indicated by the accumulation of fatty acids (FA). Myocardial FA content and enzyme release during reperfusion were correlated (r = 0.70), suggesting that membrane phospholipid degradation and cellular damage are closely related phenomena. To investigate the role of type IIA secretory phospholipase A2 (sPLA2) in this process, hearts from wild-type and sPLA2-deficient mice were subjected to ischemia-reperfusion. Postischemic functional recovery, ATP depletion, enzyme release, and FA accumulation were not significantly different between wild-type and sPLA2- deficient hearts. These findings argue against a prominent role of type IIA sPLA2 in the development of irreversible cell damage in the ischemic-reperfused murine myocardium.

PMID: 11356612 [PubMed - indexed for MEDLINE]

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1: Am J Physiol Heart Circ Physiol. 2003 Nov;285(5):H2218-24. Epub 2003 Jun 12.

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Type II secretory phospholipase A2 binds to ischemic flip-flopped cardiomyocytes and subsequently induces cell death.

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Type II secretory phospholipase A2 (sPLA2) is a cardiovascular risk factor We recently found depositions of sPLA2 in the necrotic center of infarcted human myocardium and normally appearing cardiomyocytes adjacent to the border zone. The consequences of binding of sPLA2 to ischemic cardiomyocytes are not known. To explore a potential effect of sPLA2 on ischemic cardiomyocytes at a cellular level we used an in vitro model. The cardiomyocyte cell line H9c2 or adult cardiomyocytes were isolated from rabbits that were incubated with sPLA2 in the presence of metabolic inhibitors to mimic ischemia-reperfusion conditions. Cell viability was established with the use of annexin V and propidium iodide or 7-aminoactinomycin D. Metabolic inhibition induced an increase of the numbe of flip-flopped cells, including a population that did not stain with propidiun iodide and that was caspase-3 negative. sPLA2 bound to the flip-flopped cells, including those negative for caspase-3. sPLA2 binding induced cell death in these latter cells. In addition, sPLA2 potentiated the binding of C-reactive protein (CRP) to these cells. We conclude that by binding to flip-flopped cardiomyocytes, including those that are caspase-3 negative and presumably reversibly injured, sPLA2 may induce cell death and tag these cells with CRP.

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AU Dolan R.W.; Kerr D.; Arena S.
CS Department of Otolaryngology, Boston University School of Medicine, 720 Harrison Ave.,Boston, MA 02118, United States
SO Laryngoscope, (1995) 105/12 I (1322-1325).
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CY United States
DT Journal; Article
FS 011 Otorhinolaryngology
030 Pharmacology
037 Drug Literature Index
LA English
SL English
AB Despite the known effectiveness of anti-inflammatory therapy in reducing **reperfusion injury**, no studies to date involve the use of anti-inflammatory therapy in reducing **ischemia-reperfusion injury** in fasciocutaneous flaps. Dexamethasone (a **phospholipase A2 inhibitor**) and specific cyclooxygenase and lipoxygenase **inhibitors** (indomethacin and BW755C) were administered to rats with **ischemic** island groin (fasciocutaneous) flaps. Significant improvement in **ischemic** flap survival was found with dexamethasone and BW755C. The mode of action of dexamethasone was not specifically investigated in our study; however, it may suppress neutrophil function and reduce **ischemia-reperfusion injury** in its shared ability with BW755C to reduce the formation of leukotrienes. Dexamethasone could be applied in the clinical setting to reduce **ischemic** flap loss by attenuating the systemic inflammatory response to **reperfused ischemic**-damaged tissue.
CT Medical Descriptors:
*inguinal flap
***ischemia**
***reperfusion injury**
animal experiment
animal model
article
cell viability
controlled study
drug efficacy
endothelium cell
enzyme inhibition
fasciocutaneous flap
island flap
lymphocyte activation
macrophage function
neutrophil
nonhuman
priority journal
rat
tissue injury
Drug Descriptors:
*3 amino 1 (3 trifluoromethylphenyl) 2 pyrazoline: PD, pharmacology
*dexamethasone: PD, pharmacology
*indometacin: PD, pharmacology
RN (3 amino 1 (3 trifluoromethylphenyl) 2 pyrazoline) 66000-40-6; (dexamethasone) 50-02-2; (indometacin) 53-86-1, 74252-25-8, 7681-54-1
CN Bw 755c

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AN **95306757** EMBASE
DN **1995306757**
TI The role of oxygen free radicals and **phospholipase A2** in **ischemia-reperfusion injury** to the liver.
AU Park M.-J.; Cho T.-S.; Lee S.-M.

Vol. 129 No. 8, August 1994

ARTICLE

Effects of a novel 21-aminosteroid or methylprednisolone in experimental total intestinal ischemia

P. O. Park, B. Gerdin and U. Haglund
Department of Surgery, University of Uppsala, Sweden.

OBJECTIVE: To investigate whether tirilazad mesylate, a 21-aminosteroid, protects the small intestinal mucosa from injury following total warm or cold ischemia and reperfusion. DESIGN: Randomized vehicle-controlled experimental study. SETTING: A university department of surgery. ANIMALS: Wistar rats. The warm ischemia series preceded the cold ischemia series. Animals were randomized within each series. Microscopic evaluation was performed on coded tissue slides. INTERVENTIONS: Warm ischemia was induced by a hydrostatic pressure cuff inflated to 10 mm Hg above the systolic arterial pressure for 60 minutes. Cold ischemia was studied after small intestinal transplantation. The transplant was stored for 5 hours in University of Wisconsin solution at 8 degrees C. Ischemia was followed by 60 minutes of reperfusion. In both series, tirilazad mesylate (3 mg/kg) or methylprednisolone sodium succinate (30 mg/kg) was given. Controls were given tirilazad vehicle or saline solution. MAIN OUTCOME MEASURE: Microscopic grade of small intestinal mucosal injury. RESULTS: Mucosal injury was evident in all groups of animals that were subjected to warm or cold ischemia. Reperfusion following cold ischemia induced a significant reperfusion injury. Neither tirilazad nor methylprednisolone protected the small intestinal mucosa during ischemia or reperfusion. CONCLUSION: Mucosal injury following warm or cold intestinal ischemia and reperfusion is caused by factors other than or in addition to lipid peroxidation, which is preventable by use of a 21-aminosteroid.

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1: FEBS Lett. 1998 Dec 4;440(3):377-81.

Related Articles, Link

Antibodies against type II phospholipase A2 prevent renal injury due to ischemia and reperfusion in rats.

Takasaki J, Kawauchi Y, Urasaki T, Tanaka H, Usuda S, Masuho Y.

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co. Ltd., Tsukuba, Ibaraki, Japan. takasaki@yamanouchi.co.jp

This study was performed to determine the involvement of type II phospholipase A2 (PLA2-II) in renal injury caused by ischemia and reperfusion. Ischemia and reperfusion significantly elevated levels of blood urea nitrogen and serum creatinine in rats. These increases were significantly reduced by i.v. administration of rabbit IgG F(ab')2 fragments against rat PLA2-II. Increased levels of acid-stable PLA2 activity in the kidney were caused by ischemia and reperfusion, and were suppressed by administration of anti-PLA2-II F(ab')2. Increased levels of myeloperoxidase activity, a marker of neutrophil infiltration, in the kidney were also reduced after anti-PLA2-II F(ab')2 treatment. These results suggest that PLA2-II plays a pivotal role in pathogenesis of ischemia and reperfusion injury through induction of neutrophil infiltration.

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1: J Pediatr Surg. 1998 Feb;33(2):350-3.

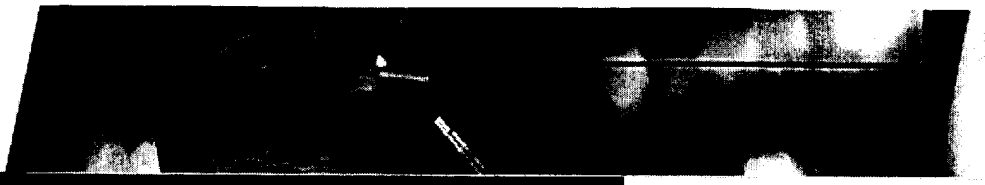
ICAM-1 upregulation in distant tissues after hepatic ischemia/reperfusion: a clue to the mechanism of multiple organ failure.

Meyer K, Brown MF, Zibari G, Panes J, McMillan RW, McDonald JC, Granger DN.

Department of Surgery and Physiology, Louisiana State University Medical Center-Shreveport, 71130-3932, USA.

BACKGROUND/PURPOSE: Endothelial cell adhesion molecules (ECAMs) are felt to play an important role in ischemia/reperfusion (I/R) injury by causing adhesion of leukocytes to endothelial cells. It is possible that ECAMs play a role in multiple organ system failure. ICAM-1 is one of the adhesion molecules that has been shown to be upregulated in response to cytokines. This upregulation leads to leukocyte endothelial cell interaction (adhesion) and to neutrophil infiltration of the affected tissue. The purpose of our study was to measure ICAM-1 expression in the liver and other organs after hepatic ischemia/reperfusion (I/R). METHODS: A laparotomy was performed on 14 Sprague-Dawley rats; 45 minutes of occlusive ischemia to the left lateral lobe was followed by 5 hours of reperfusion. The rat was injected with I125-labeled ICAM-1 MAb and I131-labeled nonbinding MAb (to control for nonspecific accumulation of ICAM-1 MAb). Entire organs were harvested and accumulated activity was measured in each organ. ICAM-1 levels were expressed as percent injected dose per gram of tissue. Control animals underwent sham laparotomy. RESULTS: ICAM-1 was upregulated in the ischemic lobe of the liver, nonischemic lobe of the liver, heart, kidney, intestine, and pancreas. Up-regulation in the lung was not significant. Both the lung and liver had high constitutive levels of ICAM-1. CONCLUSIONS: These data show that (1) significant hepatic upregulation of ICAM-1 after hepatic ischemia/reperfusion and (2) significant ICAM-1 upregulation in other tissues (heart, kidney, and intestine) after hepatic ischemia/reperfusion. The ICAM-1 upregulation in distant organs is likely mediated by cytokines such as tumor necrosis facto (TNF). These data show that leukocyte endothelial cell interactions in distant organs may be mediated by hepatic ischemia/reperfusion. This is a possible explanation for how failure of one organ can lead to failure of others in multiple organ system failure.

PMID: 9498415 [PubMed - indexed for MEDLINE]



EXPLORE HOPKINS INVENTIONS

Biological Sciences : Therapeutics

Targeting CD4+ Cells to Treat Ischemia Reperfusion Injury (JHU Ref 3931)

There is currently no specific therapy for ischemic acute renal failure. We have recently identified that CD4+ T cells directly participate in the pathogenesis of the **injury**. Furthermore, modulating CD4+ T cells improves the course of ischemic acute renal failure. With rapid developments in immunology that are now allowing for modulation of CD4+ cell function, these results have opened a new opportunity for patients with ischemic acute renal failure. Furthermore, this approach is likely to be successful in ischemic **injury** to other organs, such as **heart**, brain and **intestine**.

Inventors

Dr. Hamid Rabb, MD

Keywords

Diagnostic, antibody, immunoassay, transgenic, Medical Devices, surgical, treatment, Research Tool, animal model, antibody, reagent, Therapeutic, antibody, antiinflamatory, disease model

Patents

International Application WO 03/024399

Reference

1. Rabb H, Daniels F, O'Donnel M, Haq M, Saba S, Keane W, Tang W. Pathophysiologic role of T cells in renal **Ischemia reperfusion injury** in mice. Am J Physiol 279:F525-531, 2000

2. Burne M, Daniels F, Elgandour A, Muyeddi S, Colvin R, O'Donnell M, Rabb H. Identification of the CD4+ T cell as a major modulator of renal **Ischemia reperfusion injury**. J Clin Invest 108:1283-90, 2001

3. Rabb H. The T cell as link between innate and adaptive immune systems: implications for the **kidney**. **Kidney** Int 61: 1935-1946, 2002

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Ischemic **injury** to the **kidney** occurs during cadaveric organ transplantation. This limits the number of organs that can be used, and leads to significant problems in patients who receive transported, ischemic kidneys. Furthermore, ischemic **injury** to the native **kidney** is the leading cause of nephrology consultations in a tertiary hospital setting. Thus, these two common disease entities are ripe for application of this concept. In addition, ischemic **injury** to the **heart** is seen after myocardial **ischemia**, brain **ischemia** during stroke, and intestinal **ischemia** during hypotension. All these are potentially amenable to this novel therapeutic approach and constitute a large patient population.

Applicable Categories

- Biological Sciences » Therapeutics

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1: J Pediatr Surg. 1998 Feb;33(2):350-3.

ICAM-1 upregulation in distant tissues after hepatic ischemia/reperfusion: a clue to the mechanism of multiple organ failure.

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Department of Surgery and Physiology, Louisiana State University Medical Center-Shreveport, 71130-3932, USA.

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PMID: 9498415 [PubMed - indexed for MEDLINE]

Review article

M. KUKAN

EMERGING ROLES OF PROTEASOMES IN ISCHEMIA-REPERFUSION INJURY OF ORGANS

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Proteasomes are the main non-lysosomal, multicatalytic proteinase complexes involved in the degradation of most intracellular proteins and in numerous cell processes. Studies from isolated cell models indicate that agents that induce oxidative stress may also damage proteasomes. Similarly, continuous oxidative stress during cell aging may impair proteasome activity. In **ischemia-reperfusion** models of organ **injury**, proteasomes may be involved in several ways. First, proteasomes were found to be targets of **ischemia-reperfusion injury** of the brain and **heart**. Second, proteasome activity increased in liver models of **ischemia-reperfusion**. Third, proteasome inhibition prevented **ischemia-reperfusion injury** of the brain, **heart** and **kidney**. A major mechanism by which proteasome inhibititors may confer tissue protection is inactivation of transcription activator nuclear factor-κB resulting in a block of expression of cytokines and cell adhesion molecules during the **reperfusion** phase. Thus, proteasome inhibition represents a novel strategy for the treatment of pathologies such as stroke, infarction, and **kidney** failure.

Key words: *proteasomes, oxidative stress, **ischemia-reperfusion**, free radicals, protein oxidation*

INTRODUCTION

Interruption of blood supply to organ or tissue followed by reintroduction of blood into the affected area is called **ischemia-reperfusion injury**. The phenomenon of **ischemia-reperfusion injury** is therefore a major clinical problem after stroke, infarction, shock, organ surgery and organ transplantation. Depletion of adenosine triphosphate (ATP) and disturbance of intracellular calcium homeostasis have been suggested as major pathophysiological mechanisms during **ischemia**, leading to loss of cell viability (1,2). **Reperfusion** of ischemic tissues paradoxically exacerbates the **injury** process and leads to the release of reactive oxygen species, proinflammatory mediators and attraction of inflammatory cells infiltrating the tissues (3-5). **Reperfusion** results in a similar **injury** in several organs and leukodepletion in the **reperfusion** medium decreases tissue **injury** (6). Nuclear factor (NF)-κB appears to be a key factor of **reperfusion**-induced organ **injury** because its activation promotes the synthesis of proinflammatory cytokines and leukocyte adhesion molecules (7-9).

Poteasomes are the main non-lysosomal, multicatalytic proteinase complexes involved in the degradation of most intracellular short-lived proteins (10). They are also involved in numerous cell processes, including cell cycle progression (10), transcriptional regulation (11), generation of peptides presented on MHC class-1 molecules (12,13), stress response (14) and in regulation of apoptosis (15). Proteasomes are present in all mammalian cells (16). Investigation of the role of proteasomes in **ischemia-reperfusion** models of organ **injury** is a relatively new area of research. Since proteasome inhibition leads to inactivation of NF-κB transcriptional activity (14), it can be hypothesized that proteasome inhibitors could protect organs and tissues in **ischemia-reperfusion**-induced **injury** models. In this review the reader will first be provided with relevant information on proteasomes. Second, the role of proteasomes in oxidative stress and **ischemia-reperfusion** organ **injury** will be analyzed. Finally, crucial information regarding determination of proteasome peptidase activities has been included in the text.

KNOWN FORMS OF PROTEASOMES AND THEIR FUNCTION

Proteasomes are mainly found in cytosol, but are also present in purified nuclear and microsomal fractions (16,17). The 20S

proteasome forms a core of all proteasomes (10, 18-20). The 20S proteasome is a 700,000-dalton cylinder-shaped particle, consisting of two outer rings , made up of seven different alpha subunits. Seven ß subunits make up the two inner rings of the proteasome (*Fig.1*). The 20S proteasome has three well-characterized peptidase activities: chymotryptic-like, tryptic-like and postglutamyl peptide hydrolytic-like, which are located in the hollow cavity of the cylinder and associated with ß5, ß2, and ß1 subunits, respectively. Evidence has been reported that the 20S proteasome preferentially degrades oxidized poteins in cells undergoing oxidative stress in an ATP- and ubiquitin-independent manner (21, 22).

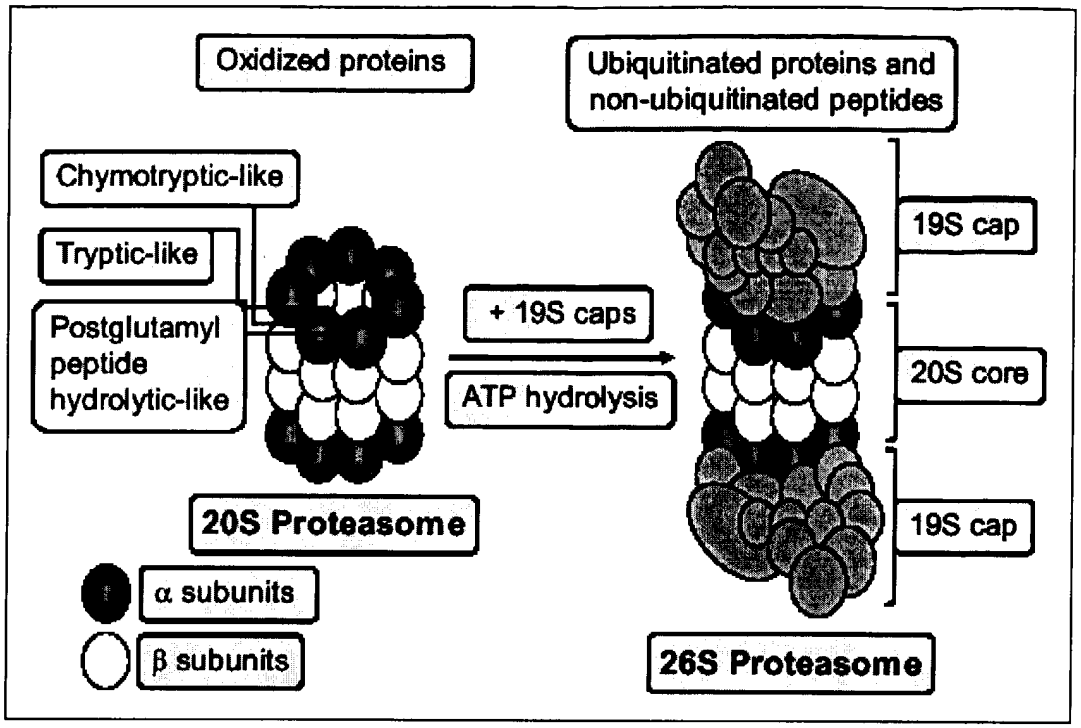

Fig. 1. Schematic presentation of the 20S and the 26S proteasome. Proteasome peptidase activities, chymotryptic-like, tryptic-like and postglutamyl peptide hydrolytic-like are associated with ß5, ß2, and ß1 subunits of the 20S core. The 26 S proteasome consists of 20S core caped with two 19S regulatory caps under ATP hydrolysis. The 19S regulatory caps recognize ubiquitinated substrates. Substrates of the 20S proteasome are oxidized proteins. Ubiquitinated proteins and non-ubiquitinated peptides are substrates of the 26S proteasome.

However, the vast majority of known protein substrates of proteasomes must be modified prior to proteolysis by covalent attachment of a polyubiquitin chain in an ATP-dependent manner (10). This process is called protein ubiquitination and it serves as a substrate-targeting and recognition signal for proteasomes. Substrate ubiquitination is accomplished by the sequential action of three enzymes: 1) an ATP ubiquitin activating enzyme, 2) ubiquitin conjugating enzyme, and 3) ubiquitin protein ligase (18). Ubiquitinated proteins are then degraded by the 26S proteasome.

The 26S proteasome is a 2,000,000-dalton complex consisting of a multicatalytic core, the 20S proteasome, and two regulatory complexes (*Fig. 1*) known alternatively in the literature as 19S cap, 19S regulatory complex, PA700, or ball (18). Formation of the 26S proteasome requires ATP hydrolysis (10). The 19S regulatory complex contains appr. 21 subunits varying in size from 25,000-110,000 daltons (23). Six subunits are a group of ATP-ase, while the remainder are non-ATP-ase subunits. Binding of 19S cap to the 20S proteasome enhances its ability to degrade ubiquitinated proteins and non-ubiquitinated peptides. Degradation of ubiquitinated proteins requires continuous ATP hydrolysis, while non-ubiquitinated peptides are degraded by ATP-independent fashion (18).

In mammalian cells, γ-interferon induces new proteasome ß-subunits: LMP2, MECL-1, and LMP7 (*Fig. 2*), which replace the respective constitutive catalytic subunits ß1, ß2, and ß5 (24, 25). γ-interferon-induced subunits have altered catalytic properties compared with constitutive subunits. These proteasomes are termed immunoproteasomes. Both the 20S and 26S form of proteasomes, as well as other forms of proteasomes, can contain LMP2, MECL-1, and LMP7 subunits (17). It appears that these subunits enhance proteasomal generation of MHC I-binding peptides (25).

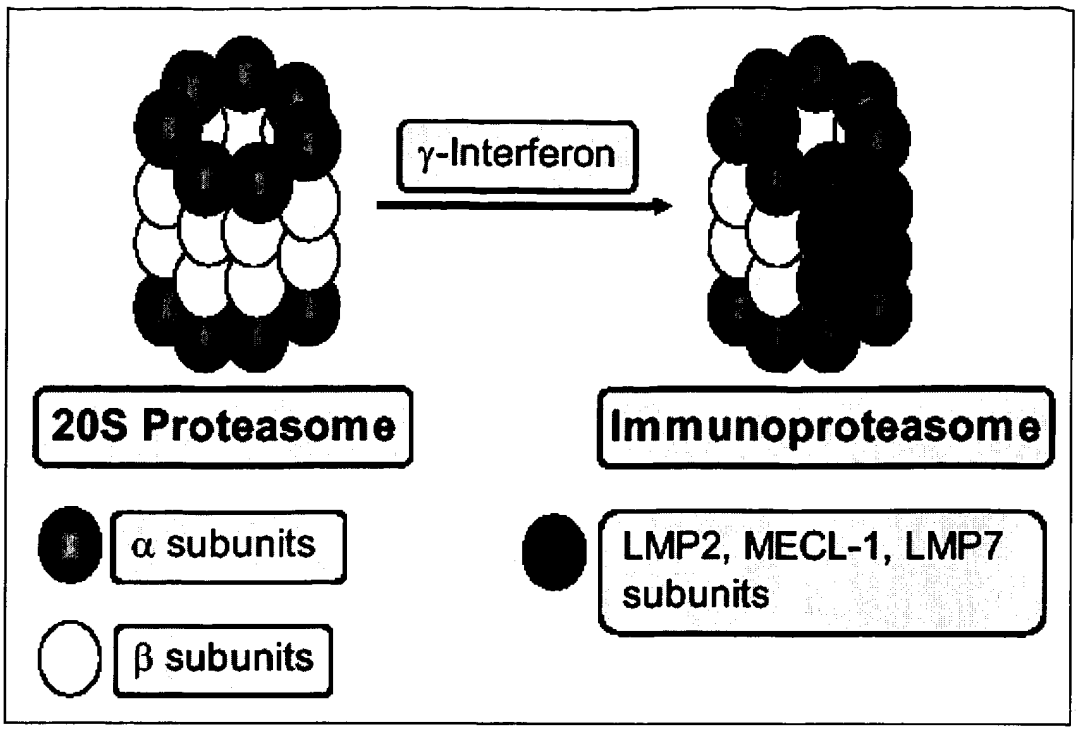

Fig. 2. Induction of proteasome subunits by γ-interferon. γ-interferon induces LMP2, MECL-1, and LMP7 subunits, which replace constitutive catalytic subunits ß1, ß2, and ß5. LMP2, MECL-1, and LMP7 subunits have altered catalytic properties compared with constitutive subunits. γ-interferon-induced subunits enhance proteasomal generation of MHC I-binding peptides.

Mammalian cells contain a proteasome activator called PA28 (also known as 11S regulator) (26). PA28 is also induced by γ-interferon. PA28 is composed of two 28,000-dalton subnits. The subunits form a ring-shaped molecule of about 180,000 daltons. In contrast to 19S cap, binding of PA28 to the 20S core does not require ATP. As for physiological function, PA28 appears to regulate the proteasome production of MHC class I molecules (27).

In adidition to the 20S and 26S, following proteasomes have been indentified:
19S-20S,
PA28-20S,
PA28-20S-PA28,
and the PA28-20S-19S, hybrid proteasome induced also by γ-interferon (18-20).

The 26S proteasome and the free 20S proteasome are major proteasomes present ubiquitously in all cells. The 20S proteasome appears to be the predominant species in mammalian cells (16).

PROTEASOMES AND OXIDATIVE STRESS

Ischemia-reperfusion injury of organs and tissues is one example of oxidative stress. Therefore, this section presents information on molecular aspects of proteasomes in oxidative stress **injury**.

Besides DNA and lipids, proteins are a major target of oxidatives stress. Oxidatively modified proteins are good substrates for the 20S proteasome while poor ones for the 26S proteasome (28, 29). Mildly oxidized proteins do not depress proteasome activity, while heavily oxidized and cross-linked proteins can inhibit proteasome function (29, 30). On using various oxidative stressors, including dopamine, ethanol, and cadmium, these agents were shown to produce significant inhibition of proteasome activity or disruption of the ubiquitin-proteasome pathway (31-33). On the other hand, impairment of proteasomal function or its blockade leads to free radical generation (34, 35).

During aerobic life organisms and cells are exposed continuously to oxidative stress. Proteasome activity or ubiquitin-proteasome efficiency decreases also with cell aging (30, 36-38). For instance, Bulteau et al. (37) found that proteasome activity in rat hearts led to loss of all three proteasome peptidase activities (shown in *Fig. 1*) with age. Moreover, these declines in activity were associated with a decreased 20S proteasome content (37). In the aged retina model, it was found that retinal proteasome from old rats exhibited a dramatic decrease in the rate of proteolysis (assessed by casein degradation) and a loss in chymotryptic-like activity. This loss in activity was accompanied by a 50% reduction in expression of the 20S proteasome (38). Interestingly, Radak and co-workers demonstrated on the other hand that resistance of animals to oxidative stress induced by exercise preconditioning was accompanied by increasing chymotryptic-like activity of the proteasome in rat brain, **heart** and muscle (39-41).

There is also good evidence that oxidative stress leading to proteasomal dysfunctions is involved in a number of degenerative

diseases (30, 34-36, 42). For instance, in Parkinson's disease, a product of the lipid peroxidation, i.e. 4-hydroxynonenal, was found to damage the 26S proteasome (34). Likewise in Alzheimer's disease, 4-hydroxynonenal was hypothesized to inhibit proteasome activity and degradation of oxidized protein substrates (42).

ISCHEMIA-REPERFUSION INJURY OF ORGANS

To our knowledge, there is only one study reporting the effect of cold **ischemia-reperfusion** on proteasome function and the effect of a proteasome inhibitor on this kind of **injury**. Therefore most **ischemia-reperfusion** studies reported here were performed at body temperature and thus mean isothermic or warm **ischemia**.

Brain

Several studies have shown that proteasome impairment occurs after cerebral **ischemia-reperfusion**. Thus e.g. Keller et al. (43) reported in the mouse model a time-dependent decrease in proteasome activity in brain tissue after cerebral **ischemia-reperfusion**. These investigators also found that mice overexpressing glutathione peroxidase displayed decreased infarct size, attenuated neurologic impairment, and reduced levels of proteasome inhibition compared with either glutathione peroxidase defective or wild type mice after cerebral **ischemia-reperfusion**. In addition, overexpression of glutathione peroxidase was associated with lower levels of 4-hydroxynonenal-modified proteasome subunits (43). These results strongly suggest that the proteasome can be impaired by both superoxide anions and 4-hydroxynonenal. Asai et al. (44) found in the gerbil model that after transient forebrain **ischemia** proteasome activity was 40% of controls in the frontal cortex and hippocampus. After 2 hours of **reperfusion**, it returned to normal levels in several regions of the brain, but remained low in the hippocampal CA1 region for up to 48 hours. When proteasome activity was assessed by incubating tissue samples in an ATP-regenerating system, 26S proteasome activity recovered almost fully in the frontal cortex but only partially in the hippocampus. These results suggest that ATP-dependent reassociation of the 20S proteasome and 19S cap to form the active 26S proteasome is specifically impaired in the hippocampus, which may lead to delayed neuronal death induced by transient forebrain **ischemia** in the hippocampal CA1 region (44). Transient inhibition of ATP-dependent conversion of the 20S proteasome to the 26S proteasome was also found in other **ischemia-reperfusion** studies and may be one of the causes of accumulation of brain tissue ubiquitin-protein conjugates in the early **reperfusion** period (45, 46).

Proteasome inhibitors block NF-κB activation and provide anti-inflammatory effects in several animal models of inflammation (47). There are also studies showing that proteasome inhibitors can afford tissue protection in brain **ischemia-reperfusion injury** (48-51). Thus e.g. in a model of middle cerebral artery occlusion, rats treated by the proteasome inhibitor CVT-634 had a significantly smaller infarct than control rats (12% vs. 20% of hemispheric volume) on day 7 of post-**reperfusion** (48). Other investigators showed that the proteasome inhibitor PS-519 administered even 2 hours after middle cerebral artery occlusion reduced the infarct volume 24 hours post-**reperfusion** by up to 60% (49). Clinical evaluations showed significant improvements in neurological function and electroencephalographic activity. Neutrophil infiltration was also significantly decreased in infarcted tissue of PS-519-treated rats. Interestingly, delayed PS-519 treatment up to 4 hours resulted also in significant neuroprotection (49). Similar neuprotective results of the proteasome inhibitor PS-519 (also called MLN519) were also found in a rat model of embolic focal cerebral **ischemia-reperfusion** (50). In a further work Williams et al. reported that MLN519 reduced proteasome blood levels by appr. 80% and decreased NF-κB activity preferentially in endothelial cells and leukocytes after middle cerebral artery occlusion-**reperfusion** in the rat (51).

Heart

All three proteasome peptidase activities (shown in *Fig. 1*) were also found to be depressed in a rat model of coronary **ischemia-reperfusion injury** (52). Moreover, analysis of the purified 20S proteasome from **heart** cytosol revealed its modification by the lipid peroxidation product 4-hydroxynonenal. However, only tryptic-like activity was markedly diminished upon proteasome purification, suggesting that **ischemia-reperfusion** may lead to production of proteasomal inhibitor(s) (52).

Similarly as documented in brain models, **heart** protection can also be achieved by proteasome inhibitors after **ischemia-reperfusion**. For instance, in the isolated neutrophil-dependent perfused rat model, Campbell et al. (53) reported an appr. 70% reduction of neutrophil infiltration in **heart** tissue treated with the inhibitor PS-519. In addition, these investigators found that PS-519 nearly completely protected coronary contractile function during the **reperfusion** period (53). Other investigators found that PS-519 protected hearts in a pig model of myocardial **ischemia-reperfusion injury** by a mechanism involving inhibition of NF-κB activation (54). Further evidence on the role of proteasomes in myocardial **ischemia-reperfusion injury** comes from studies performed with peptides PR-39 and PR-11. Both peptides have been shown to block degradation of NF-κB inhibitor $I\kappa B_{alpha}$ by the ubiquitin-proteasome pathway (55, 56). PR-11 afforded **heart** protection in a rat model of infarction (56). PR-39 peptide treatment resulted in a marked reduction of myocardial infarct size in mouse and rat models of myocardial infarction (55, 56)

Kidney

To date, proteasome activity has not yet been reported to be impaired after **ischemia-reperfusion injury** of the **kidney**. The data obtained with proteasome inhibitors strongly suggest that the proteasome pathway was involved in the pathogenesis of **ischemia-reperfusion injury** of the **kidney** (57-59). Indeed, Takaoka et al. (57) showed that before occlusion of the left renal artery administration of PSI, a proteasome inhibitor, abolished decreases in renal function of acute renal failure rats after the **reperfusion** period. Moreover, histopathological evaluation of the **kidney** of untreated acute renal failure rats revealed severe renal lesions, which were significantly suppressed by PSI treatment (57). In another study, PSI was also shown to ameliorate renal function in ischemic renal failure rats, which was accompanied by a decrease in renal endothelin-1 production (58). Since calpeptin, a calpain inhibitor, had minimal effect on endothelin-1 production and on renal function, it was proposed that a proteasome-dependent proteolytic pathway must have been involved in the enhanced production of endothelin-1 in the **kidney** and the consequent renal functional

damage in ischemic acute renal failure rats (58). Lactacystin, another proteasome inhibitor, abolished the effect of **ischemia-reperfusion** renal **injury** on endothelin-1 production 2 and 6 hours post-**reperfusion** (59). Again, the decrease in endothelin-1 production was associated with improved functional parameters of the **kidney** as well as with improved histopathological findings in lactacystin-treated rats.

Liver and other organs and tissues

Effects of **ischemia-reperfusion injury** of the **liver** (on the level of the whole animal) on proteasome activity were studied only in the turtle. Although all three proteasome peptidase activities (shown in *Fig. 1*) were found to be present in the **liver** of this species, postglutamyl peptide hydrolytic-like activity was most abundant (60). After exposure of the turtle to **ischemia** for 20 hr (submergence in N_2-bubbled water) and subsequent 24-hr aerobic recovery, the activity of this enzyme increased by one third. The authors concluded that elevated postglutamyl peptide hydrolyzing activity during recovery may serve to remove proteins damaged by oxygen free radicals generated after reintroduction of oxygen (60). In a model of cold **ischemia-reperfusion injury** of rat **liver** endothelial cells, chymotryptic-like activity was doubled. Further, cell **injury** and apoptosis were strongly inhibited by the proteasome inhibitor 3,4-dichloroisocoumarin, suggesting that the proteasome may be involved in cold **ischemia reperfusion**-induced apoptosis of **liver** endothelial cells (61). Regarding the role of proteasomes in other models of **ischemia-reperfusion injury** of organs and tissues, such as the lungs, intestines, pancreas, muscle, no information has come to our attention in the literature.

DETERMINATION OF PROTEASOME PEPTIDASE ACTIVITIES

Findings presented in the foregoing sections of this paper point to proteasomes as targets of **ischemia-reperfusion injury** (43, 44, 52). In this model of oxidative stress, proteasome peptidase activities may be increased (60, 61). Therefore, future **ischemia-reperfusion** studies should be designed so as to allow determination of proteasome activity in tissue samples.

Proteasome peptidase activities can be assessed by monitoring the release of fluorophor (e.g., 7-amino-4-methylcoumarin or ß-napthylamine) from peptide substrates. Substrates for individual proteasome sites are shown in *Fig. 3*. Incubation conditions of peptide substrates with tissue samples were reported for the brain (43, 44), blood (62), **heart** (52), **kidney**, **liver**, small **intestine**, skeletal muscle and the brown adipose tissue (63). It must be stressed however that a very recent study questioned previous measurements of proteasome activity in crude tissue samples (64). Indeed, it was found that other proteinases can degrade proteasome peptide substrates. Moreover, proteasomal inhibitors did not completely inhibit proteasome activity in crude tissue and cell lysate samples (64). In addition, several recent studies also showed that lactacystin, a highly specific inhibitor of the proteasome (65), inhibited other proteinases (66-68). In light of these findings it is not recommended to interprete proteasome activity as proteasome-inhibitable. To overcome pitfalls in determining proteasome peptidase activities it is advisable to filter tissue samples using 500 kDa membranes prior to measurements (for details see ref. 64).

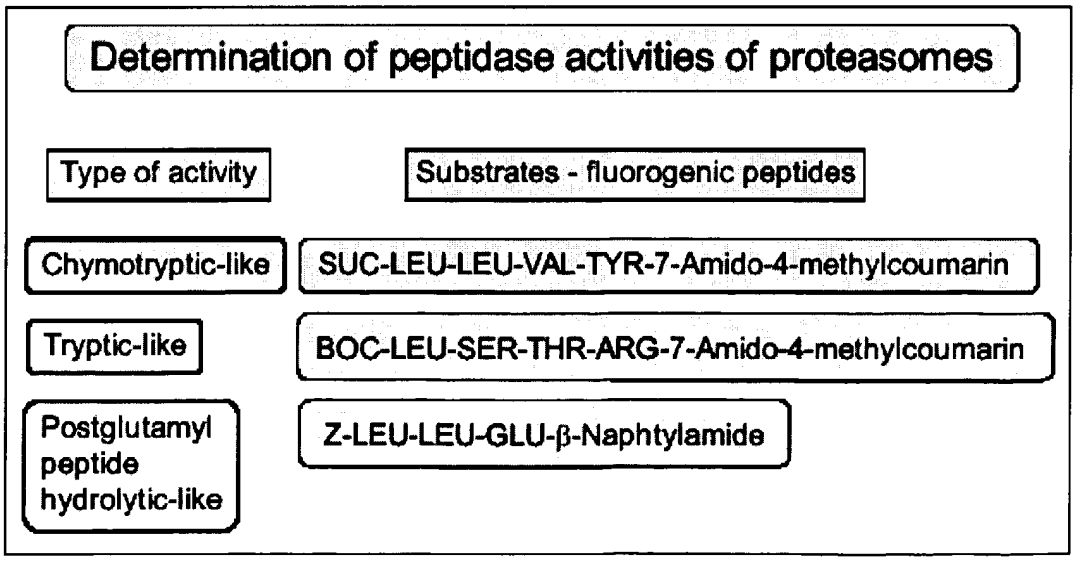

Fig. 3. Individual proteasome peptidase activities can be assessed with fluorogenic peptides substrates shown in the figure. (Enzyme units can be expressed as mol 7-amino-4-methylcoumarin/min or ß-napthylamine/min.)

CONCLUSIONS

There is emerging evidence indicating that proteasomes may be both targets and actively involved in models of **ischemia-reperfusion** organ **injury**. Thus, a comprehensive picture about the effect of **ischemia-reperfusion** on a specific organ or tissue with regard to proteasomes would be obtained only by performing both proteasome inhibitor studies and proteasome activity measurement. Studies of proteasome inhibitors in the brain, **heart** and **kidney** warm **ischemia-reperfusion** models suggest that stabilization of the IκB-NF-κB complex may be a mechanism by which proteasome inhibitors protect organs and tissues during the **reperfusion** phase. Involvement of proteasomes in other **ischemia-reperfusion** models of organ **injury** have not been reported as

yet and thus provide a broad area for future research.

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